Neural cell patterning on thin film boron doped diamond electrodes for neurotransmitter detection *in vitro*

Jeannette M. Bursee, Harihara Baskaran, and Heidi B. Martin

Department of Chemical Engineering

Case Western Reserve University, Cleveland, OH 44106

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Abstract

We present our progress in the development of an *in vitro* method to measure neurotransmitter release and electrical signals from neural cells by patterning the cells directly onto an array of diamond electrodes and by controlling the growth of neural processes in culture. The experimental model allows quantification of electrochemical communication between neural cells, and provides a more predictable system for the study of electrochemical communication between neurons than *in vivo* animal models or studies using brain slices. Using various microfabrication techniques, it is possible to achieve small feature sizes enabling observations at the cellular level. Detection with extracellular electrodes provides a non-invasive method to detect chemical and electrical signals from cells.

Cell patterning techniques provide a convenient method to control the growth of cells and for strategic placement of neurons in culture over an electrode surface to facilitate neurotransmitter detection. Self-Assembled Monolayers (SAMs) of alkanethiolates on gold substrates produced via microfabrication techniques have been used to provide a pattern of adhesive and nonadhesive regions which may be used to control cell growth over a substrate. Application of various patterns of adhesion proteins over a substrate can be achieved using microfluidic techniques or elastomer stamps. Preferential cell adhesion to these proteins can be used to create patterns of cells, and to control axon and neural process growth. [1,2] The terminal functional groups of the diamond thin film determine hydrophobicity properties and possibly cell adhesion properties; this may provide a possible avenue for cell patterning as well. Neural cells have been previously patterned over electrode arrays in order to detect electrical signals produced by these cells, *e.g.*, patterns over gold electrode contacts and commercially available microelectrode arrays. [3,4] Thus far, the technique of cell patterning to control neural process growth has not been applied to study the spatiotemporal dynamics of the release of particular neurotransmitters at the synapse between two neurons.

Diamond has gained interest as an electrode material for neurological measurements. Boron-doped diamond electrodes have been shown to have a wide potential window and low baseline current in aqueous solutions and provide long-term chemical stability. [5] Diamond electrodes potentially provide an improved sensor material for *in vivo* detection of neurotransmitters. Carbon-fiber electrodes are used widely in neurochemical sensing research, however they do not display the same long-term stability as thin film diamond electrodes; the surface becomes passivated. Protein adsorption onto the surface of carbon-fiber electrodes also decreases their functionality over time. One possible solution to this problem is to coat the electrode surface with a porous conducting layer such as Nafion, but this can limit the sensor response time.

Diamond has a simpler surface chemistry than carbon fibers, possibly enabling homogenous, surface functionalization. Diamond's surface termination can thus be more predictably modified to manipulate the electrochemical properties. The diamond surface may also discourage the adsorption of proteins, which is desired for continuous detection of biomolecules in aqueous solution. [6,7]

As the first steps to develop this diamond device to probe neural cell activity, we are developing techniques for diamond and protein patterning, as well as studying interactions of cells with these surfaces. To fabricate diamond electrode patterns, we used CVD differential growth of diamond onto a resistive silicon substrate. Prior to growth, microfluidic techniques distributed diamond seed nanoparticles into a precise pattern (minimum width of pattern ~ 200 μm, smaller microfluidic feature sizes down to ~20μm may be fabricated using current method). Initial results show that seeded regions developed nearly complete films and had smaller crystals than regions where seed particles were not deposited. Areas where no nanoparticles were applied showed incomplete film formation. The pattern of diamond film formation can be seen in Figure 1. Similarly, the conductance of the more complete diamond film in the pre-seeded regions was significantly higher than in non-seeded areas. (~55 μSiemens vs. ~2.0

μSiemens). We are currently varying the deposition time, concentration of seed particles, and the geometry of the microfluidic patterns to optimize this process.

Preliminary studies with a cancer cell line on a diamond thin film over a silicon substrate show that eukaryotic cells adhere to a diamond thin film and undergo cell division. The cells were also cultured on the diamond film pattern. The cells adhere preferentially to the more complete areas of film growth (*i.e.*, pre-seeded regions).

Coupled with our diamond patterning is development of protein patterns. In general, a culture of cells may be induced to grow in specific geometries by differential application of adhesion proteins to a substrate. [8] We are currently applying a pattern of the cellular adhesion protein, fibronectin, to the substrate using microfluidic techniques. The protein pattern is visualized by fluorescently labeling the fibronectin through a standard biotinylation process and reaction with fluorescently conjugated streptavidin. Time-lapse microscopy is then used to determine the differential time for adhesion of the cells to the protein pattern and the unpatterned substrate.

In the future, we will integrate our patterning techniques, to pattern cells from a human neuronal cell line over the diamond thin film electrode array such that release of neurotransmitters from specific groups of neural cells may be detected at an electrode surface.

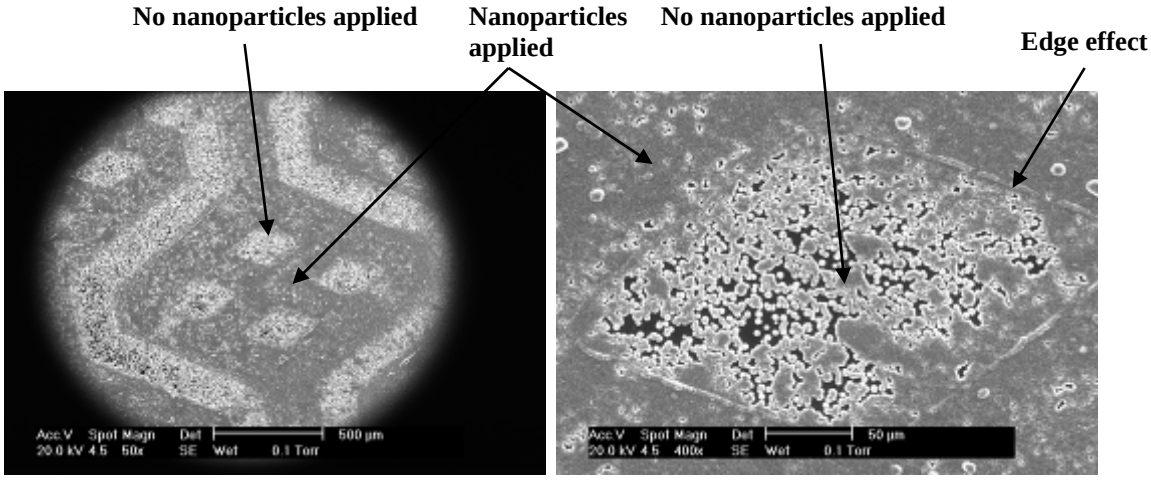

Figure 1. Diamond seed particles were applied to a resistive silicon substrate using microfluidic techniques. Areas where diamond seed nanoparticles were applied (these areas appear darker) showed nearly complete film formation and had a smaller crystal size than where seed particles were not deposited. Areas where no nanoparticles were applied showed incomplete film formation and had a larger crystal size than. The conductance of the areas where seed particle were applied was significantly higher than where seed particles were not applied. (~55 μSiemens vs. ~2.0 μSiemens). Image on left, scale bar = 500 μm, on right, scale bar = 50 μm.

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